Shredders and leaf breakdown in streams polluted by coal mining in the South Island, New Zealand.

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Abstract

Leaf breakdown and macroinvertebrate colonisation of artificial leaf packs were investigated in six streams experiencing coal mine discharge and metal deposition near Reefton, South Island, New Zealand. Nine sites; three with metal precipitates prominent on their beds, three with abundant metal bacterial flocs and three control sites with no mine related discharges were investigated over a nine week period during summer. pH was lowest in streams dominated by metal precipitates (pH 3.8 – 4.6), which also had the highest concentrations of dissolved iron (1.16 ± 0.44 g / m^3) and dissolved aluminium (1.83 ± 0.96 g / m^3). Leaf breakdown of American sycamore (*Platanus occidentalis*) was slow in all streams ($-k$/day = 0.0007 – 0.007) and did not differ significantly among stream types. Leaf toughness took longer to decline in streams dominated by metal precipitates than in streams with metal flocs suggesting that leaves would have taken longer to decompose in precipitate streams. Shredder densities were low in all streams (< 4 per leaf pack) and precipitate streams had the lowest macroinvertebrate densities per leaf pack (< 6). Our findings indicate that organic matter processing may be reduced only marginally in streams dominated by metal flocs, however metal precipitates that can form on leaves in mine drainage streams may increase the time taken for leaves to soften and hinder the processing of organic matter.

Keywords: Leaf breakdown - macroinvertebrates - acid mine drainage - metal deposition - streams.

Introduction

In the South Island of New Zealand coal mining frequently occurs in forested regions where stream food webs are subsidised by organic matter that falls or is leached into streams. The conversion of particulate organic matter (i.e., leaves and woody debris) occurs primarily by physical abrasion, microbial decomposition and the activities of invertebrate shredders (Webster & Benfield 1986). In streams affected by mine discharges both microbial activity and shredding by invertebrates can be greatly reduced (Maltby & Booth 1991, Griffith & Perry 1993, Siefert & Mutz 2001).

Most research into the effects of acid mine drainage (AMD) on stream ecosystems has focused on changes to benthic invertebrate communities due to lowered pH and increased concentrations of heavy metals. However, stream communities affected by AMD are not influenced solely by changes in water chemistry. In fact, stream biota often have to contend with changes to their physical environments caused by metal precipitates (e.g., iron and aluminium). In particular, iron hydroxide (FeOH) precipitates are often associated with streams impacted by coal mining (Soucek *et al.* 2003, Harding & Boothroyd 2004). Iron precipitation is predominantly a consequence of the pH-dependent oxidation of soluble ferrous iron (Fe^{2+}) to less soluble ferric iron (Fe^{3+}) (Broshears *et al.* 1996). In streams with pH < 3 iron generally remains in its soluble form (Harding & Boothroyd 2004); however, dilution of AMD waters by heavy rainfall and downstream tributaries can lead to an increase in the pH and subsequent precipitation of iron from the water column on to the surrounding river bed (Kim & Kim 2004). Iron deposition can also be in the form of blooms or flocs of iron-depositing bacteria (e.g., *Leptothrix* and *Sphaerotilus*). These bacteria are widespread in nature and can occur in freshwaters with near-neutral pH (Ghiorse 1984) as well as those impacted severely by acid mine drainage (Ferris *et al.* 1989). Although the direct involvement of sulphur- and iron-oxidising bacteria in the formation of AMD is well known (McGinness & Johnson 1993, Bond *et al.* 2000, Johnson & Hallberg 2003), the role of bacteria in the formation of iron flocs can be complex (Clarke *et al.* 1997, Crundwell 2003, Kappler & Newman 2004). Thus, mine drainage can exert a range of stresses on a stream ecosystem, stresses that can act individually, or collectively, to modify in-stream processes such as leaf breakdown.

Leaves and wood that enter streams are significant sources of energy for stream communities and can provide important foods and habitat resources for macroinvertebrates (Fisher & Likens 1973, Petersen & Cummins 1974, Anderson & Sedell 1979). Leaf breakdown includes at least three distinct phases after entering a stream: leaching, conditioning and fragmentation (Cummins 1974, Petersen & Cummins 1974). In the second of these stages, leaves undergo conditioning, which involves the colonisation of leaf surfaces by micro-organisms (bacteria and fungi) that begin the decomposition process and increase the palatability of leaves to detritivorous macroinvertebrates. However, the roles of bacteria, fungi and macroinvertebrates in litter breakdown can be influenced by a variety of anthropogenic stresses including the lowering of stream water pH, increased concentrations of dissolved heavy metals, and the deposition of metal oxides (Kelly 1988).

In streams receiving mine drainage, leaf breakdown is often slower than in 'non-impacted' streams (Carpenter *et al.* 1983, Maltby & Booth 1991), and maybe a consequence of changes to populations of sensitive macroinvertebrates, fungi, or bacteria. For example, some fungi, bacteria, and macroinvertebrates are sensitive to low pH (Townsend *et al.* 1983, Allard & Moreau 1986, Palumbo *et al.* 1987, Smith *et al.* 1990), heavy metals (Clements *et al.* 1988, Rasmussen & Lindegaard 1988, Niyogi *et al.* 2002) and metal deposition (McKnight & Feder 1984, Niyogi *et al.* 2001).

Reductions in microbial respiration and production have been observed in low pH waters (Palumbo *et al.* 1987) and have been linked to slower organic matter decomposition rates (Allard & Moreau 1986). Reductions in microbial activity may also lead to a reduction in food quantity and quality for detritivorous invertebrate species (Townsend *et al.* 1983). Furthermore, organic matter breakdown may be particularly slow in streams suffering metal deposition. Gray & Ward (1983) observed that ferric hydroxide deposition on leaves, directly inhibited the colonisation of both fungi and invertebrate shredders.

The aim of the present study was to compare the initial stages of leaf breakdown and colonisation of leaf packs by macroinvertebrates among streams dominated by metal precipitates, metal bacterial flocs or those unaffected by AMD.

Methods

Study area

Our study was conducted near Reefton on the West Coast of the South Island, New Zealand (Figure 1). Nine sites on eight streams were selected for study. Six sites were affected to varying degrees by mine pollution and metal deposition as a result of past and present coal mining operations. They included three sites on three streams with beds coated by metal flocs: Old Terrace Mine Stream (F1), Burke Creek (F2) and a tributary of Murray Creek (F3). A further three sites affected by metal precipitates were located on Progress Creek (P1), Garvey Creek (P2) and Wellman Creek (P3). Sites were classified visually as the mechanisms controlling the presence of metal precipitates or metal bacterial flocs was not investigated. Finally, three sites unaffected by mining discharges were located in upper Burke Creek (R1), a tributary of Murray Creek (R2) and Devils Creek (R3). R3 was approximately 2.5 kms downstream from Progress Creek, which had no apparent effect on the water chemistry of R3.

Water quality

Temperature, specific conductivity, and pH were measured in the field on three occasions between December 2004 and February 2005, using an Oakton CON 10 Series meter. Turbidity (HACH 2100P Turbidimeter), was also measured one to three times in the laboratory on shaken water samples. Grab water samples were taken on one occasion from eight of the nine sites and analysed for dissolved iron, aluminium, arsenic, zinc and nickel at a commercial laboratory using atomic absorption spectrophotometry (RJ Hills Laboratory, Hamilton, New Zealand). Detection limits for these dissolved metals were: iron (0.02 g / m^3), aluminium (0.003 g / m^3), arsenic (0.001 g / m^3), nickel (0.0005 g / m^3) and zinc (0.001 g / m^3).

Leaf breakdown

Abscised American Sycamore (*Platanus occidentalis*: Platanaceae) leaves were collected from Christchurch in early May 2004 and returned to the laboratory where they were dried at room temperature and stored until use. One hundred and eight, 5 g dry weight leaf packs were constructed using the dried American Sycamore leaves, which were placed into 5 mm-mesh nylon onion bags. To prevent physical abrasion the bags of leaves were placed in PVC canisters (30

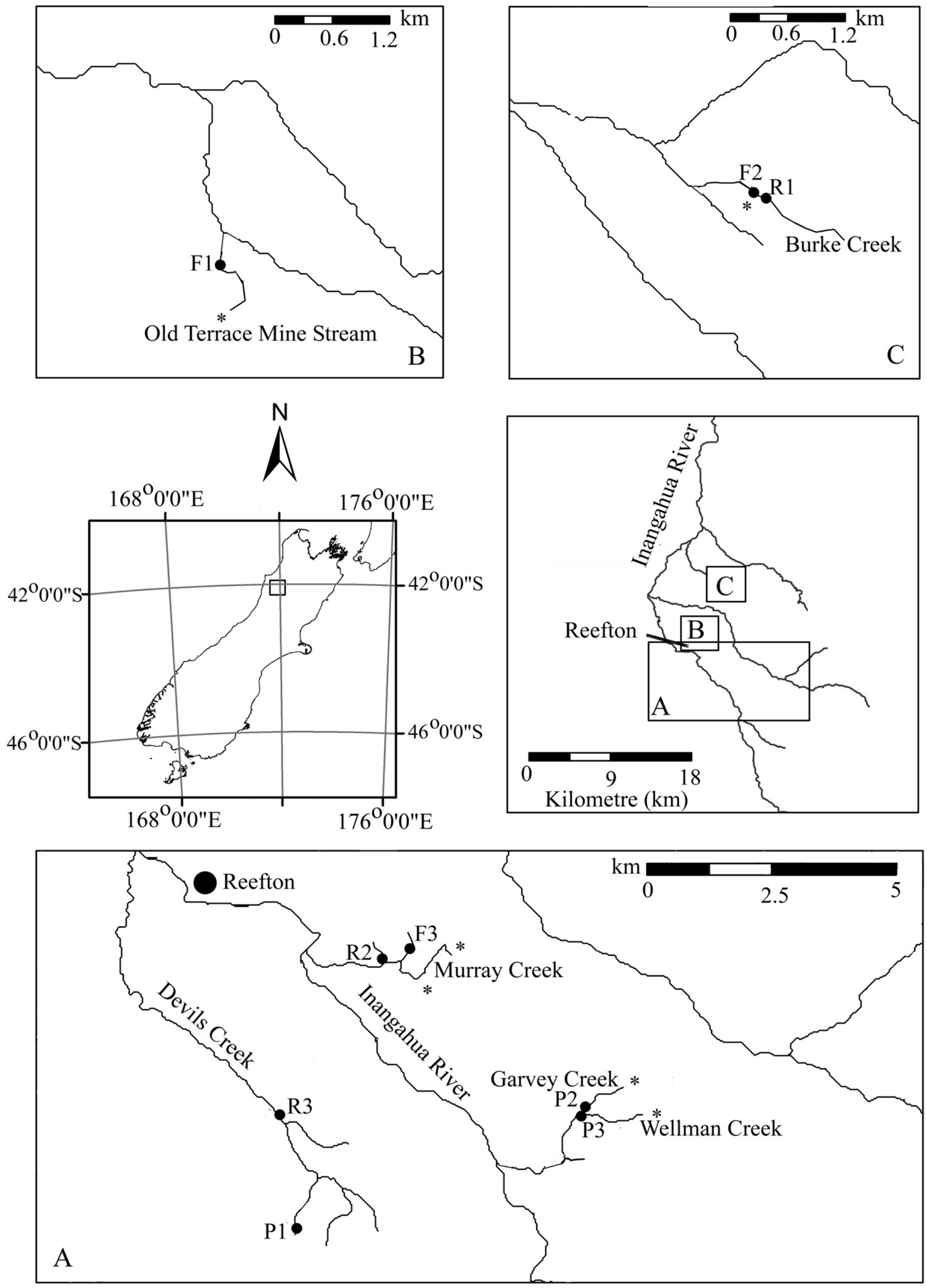

Figure 1. Location of sampling sites within the study area near Reefton, South Island, New Zealand. * denotes approximate location of known coal mining operations.

cm length) with 1 cm diameter holes drilled along their lengths to permit water flow and invertebrate colonisation. Caps were attached to the ends of the canisters to prevent loss of leaf packs. Two canisters, each containing six leaf packs were submersed and secured to the streambed at each of the nine study sites on 23 December 2004. Three leaf packs were recovered from each site after two, five, seven and nine weeks. Recovered leaf packs were placed in separate zip-lock plastic bags and stored on ice in a chilly bin for transport to the laboratory where they were frozen until analysis.

In the laboratory, leaves making up each pack were thawed and washed through a 500 µm-mesh sieve to remove fine debris, attached sediments and invertebrates. They were then tested for toughness with a penetrometer (Feeny 1970, Quinn *et al.* 2000). Penetrance is a relative measure of leaf toughness, and is defined as the weight required to force a blunt rod through a leaf (Young *et al.* 1994). To do this we recorded the weight of lead shot (g) needed to force a 0.785 mm^2 diameter rod through a wet leaf. Fifteen measurements were made on leaves randomly drawn from each leaf pack. Veins were avoided. Penetrance pressure (PEN, kPa) was calculated after Quinn *et al.* (2000) using the formula 9.807 M / 0.785 (where 9.807 is gravitational force, M is the applied mass needed to pierce the leaf and 0.785 is the area of the rod). The weight of the penetrometer stand was 22 g and thus the kPa was 275. When the rod penetrated the leaf without applying any additional weight half this penetrance value was recorded (Quinn *et al.* 2000). Young *et al.* (1994) suggested that freezing may alter leaf penetrance, but because all leaf packs received the same treatment this would have affected all leaves equally.

All leaf pack material was placed into labelled containers and dried at 40° C for 96 h, after which it was weighed to the nearest 0.001 g. The average dry weight (± 1SE) of leaves remaining at each site on each occasion was calculated from the three leaf pack weights.

Macroinvertebrates were hand picked from leaf packs and identified under 40x magnification. Macroinvertebrates were identified to the lowest possible taxonomic level: usually genus or species, except for Chironomidae, which were identified to subfamily and Oligochaeta, Ostracoda, Acarina and Collembola, which were not identified beyond order or class. Identifications of insects were made using Winterbourn *et al.* (2000). Macroinvertebrates were assigned to functional feeding groups in consultation with M.J. Winterbourn.

Data analysis

In all analyses stream sites were used as replicates and plots of residuals versus fits and normality plots were used to test for normality and homoscedasticity of data. Where assumptions of normality and homoscedasticity were not met response variables were log transformed (x + 1 where necessary) (Zar 1999).

To determine whether water chemistry parameters and heavy metals differed among the three stream types one-way analyses of variance (ANOVAs) were used. Differences detected by ANOVAs were tested using Tukey's post-hoc test (HSD). We used repeated measures ANOVA to test the effects of stream type and time (weeks) on leaf toughness, the number of taxa in leaf packs, the number of invertebrates per gram leaf pack, and the number of shredders per gram leaf pack. Mean values for each stream were used as replicates.

The rate of leaf breakdown (k / day) in each stream was modelled as a negative exponential function (Petersen & Cummins 1974) using the dry weight of the leaf packs:

$$- k \text{ day} = \log_e (\% \text{ R} / 100) / \text{t}$$

where, % R = dry weight of leaf remaining after 63 days, and t is time in days. We used a one-way ANOVA to test for differences in leaf breakdown rates between stream types.

Results

Water quality

Median pH and pH range were lower and conductivity was significantly higher in precipitate streams than in flocculent and reference streams (Table 1). Precipitate streams had higher concentrations of dissolved iron, aluminium, nickel and zinc than reference streams but a significant difference among stream types ($P < 0.05$) was found only for iron, which was higher in flocculent and precipitate streams (Table 1). A boarderline significant difference ($P = 0.051$) was found for aluminium, which was highest in precipitate streams.

Leaf breakdown

Leaf weight loss was similar across stream types (Figure 2a). Breakdown rates (k) found among the stream types ranged from 0.003 – 0.007 in the flocculent streams to 0.0007 – 0.002 in the precipitate streams, and 0.002 – 0.008 in the reference streams and were not statistically different (One- way ANOVA, $F_{2, 6} = 2.555$; $P = 0.157$). All k values indicated slow rates of leaf breakdown (Petersen & Cummins 1974).

A repeated measures ANOVA indicated that leaf toughness did not differ among the three stream types (Table 2), however, there was a time effect indicating that leaf toughness declined with time in the stream (Figure 2b). Furthermore, a significant stream type by week interaction (Table 2) indicated that the rate of decline in leaf toughness differed among stream types over time. Leaf toughness declined in a linear manner

Table 1. Summary of water chemistry values (± 1SE, except pH) in the three stream types in summer (2004 – 2005). Results of one-way ANOVA are also shown with significant results ($P < 0.05$) in bold. Stream type differences are indicated by different superscript letters.

	Flocculent	**Precipitate**	**Reference**	$F_{2,6}$	***P***
Water chemistry					
pH median	6.8	4.2	7.1		
pH range	5.5 - 6.8	3.8 - 4.6	6.7 - 7.5		
Conductivity (μS_{25} / cm)	70.5 ± 4.3 ^{a}	460 ± 197 ^{b}	7.3 ± 22.6 ^{a}	9.11	**0.015**
Turbidity (NTU)	14.3 ± 6.17	5.2 ± 2.2	7.0 ± 3.50	1.23	0.356
Temperature (°C)	3.8 ± 0.87	13.4 ± 0.8	5.7 ± 0.54	2.7	0.143
n	*3*	*3*	*3*		
Dissolved metals (g / m³)				$F_{2,5}$	***P***
Iron	0.32 ± 0.013 $^{a, b}$	1.16 ± 0.44 ^{b}	0.17 ± 0.11 ^{a}	3.02	**0.047**
Aluminium	0.15 ± 0.03	1.83 ± 0.96	0.13 ± 0.09	5.2	0.051
Arsenic	0.002 ± 0.001	0.001 ± 0.0	0.013 ± 0.011	0.79	0.503
Nickel	0.003 ± 0.006	0.044 ± 0.003	0.001 ± 0.006	2.15	0.211
Zinc	0.010 ± 0.001	0.160 ± 0.14	0.002 ± 0.001	2.37	0.188
n	3	2	3		

over the first seven weeks at the reference sites, whereas leaves at the flocculent sites became softer more slowly. However, after week seven their toughness declined rapidly, and by week nine they had similar toughness values to reference stream leaves (Figure 2b). In contrast, leaves at precipitate sites showed little change in toughness over the first seven weeks (and in some cases may even have become tougher), a condition that was mirrored by the appearance of iron encrustments

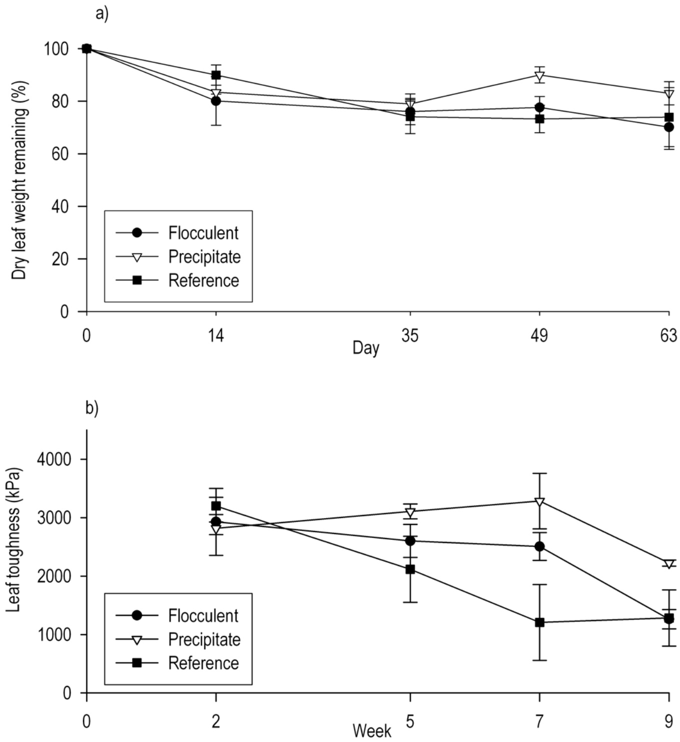

Figure 2. Mean a) percentage of dry leaf weight remaining in packs submersed in three types of streams over 9 weeks in summer (2004 – 2005), and b) changes in leaf toughness over time in the three stream types (n=3, ± 1SE).

Table 2. Results of repeated-measures ANOVAs comparing leaf toughness and colonisation of packs by invertebrates across three stream types over nine weeks. *F* values and significance shown, where * *P* < 0.05, *** *P* < 0.001, ns = not significant.

	Toughness	No. of taxa	No. of invertebrates	No. of shredders
Between subjects				
Stream type	2.79 ns	2.82 ns	1.87 ns	8.34 *
Within subjects				
Week	11.04 ***	3.03 ns	1.98 ns	2.62 ns
Stream type: week	3.02 *	1.66 ns	0.18 ns	1.55 ns

on leaves.

Macroinvertebrates

Although average numbers of taxa were 1.5 to three times higher in leaf packs from reference streams than precipitate streams (Figure 3a), taxon number did not differ significantly among stream types (Table 2). Furthermore, taxon richness did not change significantly ($P > 0.05$) over time, and no interaction effect was found between stream types and time (Table 2). Invertebrate densities were up to ten times higher in leaf packs from reference streams than precipitate and flocculent streams (Figure 3b) but variation among reference streams was high so densities did not differ significantly among stream types (Table 2).

Few shredders were collected in leaf packs from any of the stream types and none were found in leaf packs from precipitate streams (Figure 3c; Table 2). The only shredders collected in leaf packs from reference sites were larvae of the stonefly, *Austroperla cyrene* and small hydraenid beetles. *A. cyrene* was also collected in leaf packs from flocculent sites where larvae of the caddisfly *Triplectides obsoletus* was also collected. No significant difference in shredder abundance was detected among weeks and no interaction effect was found between stream type and time (Table 2).

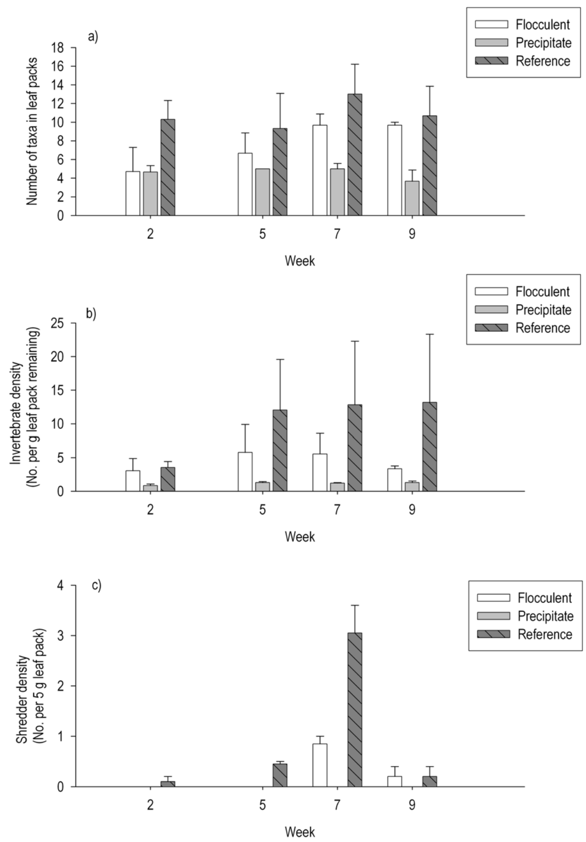

Figure 3. Mean a) taxonomic richness, b) number of invertebrates and, c) number of shredders in leaf packs in the three stream types on four sampling occasions (n=3, ± 1SE) in summer (2004 - 2005).

Table 3. The five numerically dominant taxa collected in leaf packs in the three stream types in summer (2004 – 2005). Functional feeding group (FFG) designations for each taxon are shown, where CB= Collector-browser, P=Predator. Numerals indicate ranked abudances.

		Dominance in stream types		
Taxa	**FFG**	**Flocculent**	**Precipitate**	**Reference**
Trichoptera				
Pycnocentrella eruensis	CB	1		4
Diptera				
Chironominae	CB	2	3	1
Orthocladiinae	CB	3	1	3
Tanypodinae	P		4	5
Eriopterini	CB	5		
Plecoptera				
Spaniocerca	CB	4	5	2
Coleoptera				
Scirtidae	CB		2	

Leaf packs in flocculent and reference streams were dominated by a cased caddisfly, three dipterans and a stonefly, whereas leaf packs in precipitate streams were dominated primarily by dipterans (Table 3). These dominant taxa were all collector-browsers or predators.

Discussion

We anticipated that within the nine weeks of this study there would be a detectable difference in the rate of leaf breakdown among the three stream types. However, our results indicated no difference in breakdown rates. Nevertheless, changes in leaf toughness suggested that leaves in reference streams may have broken down faster than in mine impacted streams (precipitate and to some extent flocculent streams) over a longer period of time. The rate of leaf breakdown (k) in all our streams was comparable to the slower rates reported by Sponseller & Benfield (2001) for American Sycamore leaves in North American streams with few shredders, and by Benfield *et al.* (1977) in a pastureland stream.

Rates of leaf breakdown in streams are influenced by several factors, including variation in water flow, water quality, and inorganic sediment deposition on leaves (Suberkropp & Chauvet 1995, Maamri *et al.* 1997, Schlief 2004). Water chemistry varied among our sites and we had anticipated that differences would be reflected in the speed of leaf breakdown. However, despite the flocculent sites, and especially the precipitate sites, having lower pH, and elevated levels of iron, aluminium, zinc and nickel, leaf breakdown rates were similar to those at the reference sites. The results of our study therefore differ from those of Allard & Moreau (1986), who showed that in artificially acidified streams, leaf decomposition could be markedly reduced in water at pH 4.0 compared to pH 6.2 - 7.0. They suggested that the lower rate of leaf breakdown was a consequence of a reduction in microbial activity in the acidified waters, rather than a reduction in the numbers of macroinvertebrates. Similarly, Bermingham *et al.* (1996) found that leaf decomposition and associated microbial activity, particularly that of fungi, was markedly reduced in a stream receiving coal mining effluent with elevated iron and nickel, relative to non-impacted, upstream control sites. Bermingham *et al.* (1996) used a mesh size that excluded macroinvertebrates from their leaf packs, and concluded that the slower leaf breakdown was largely due to the reduction in fungal activity brought about by elevated concentrations of heavy metals. In contrast, Collier & Winterbourn (1987) concluded that the faster rate of breakdown of *Weinmannia racemosa* leaves in clear water streams (pH 6.6 – 8.0) than brown water streams (pH 4.3 – 5.7) was due largely to the presence of invertebrate detritivores, which were rare in the brown water streams.

The deposition of inorganic sediment on leaves may also reduce the rate at which they breakdown and Gray & Ward (1983) concluded that the deposition of ferric hydroxide on leaves inhibited colonisation by microbes and invertebrates leading to slower decomposition. Leaves incubated in our precipitate streams appeared to incorporate FeOH into their tissues and remained tougher than leaves in the flocculent and reference streams throughout the nine weeks of this study.

Other studies have linked differences in leaf conditioning (or leaf softening) to temperature, effects of pH on enzyme activity, and the nutrient concentrations of leaves and / or the surrounding water (Suberkropp & Klug 1980, Young *et al.* 1994, Jenkins & Suberkropp 1995, Molinero *et al.* 1996, Quinn *et al.* 2000, Niyogi *et al.* 2003, Woodcock & Huryn 2005). Generally, higher rates of softening occur at high temperatures, in alkaline waters, and in nutrient rich leaves and / or stream water. In our study we detected no difference in temperature between the three stream types, and as all sites were in forest and geographically close to each other they would not be expected. Although, we did not measure nutrient concentrations at our study sites, Anthony (1999) reported no significant differences in the concentrations of nitrate-nitrogen and reactive phosphate in mine drainage and non-impacted streams in the same study area. Furthermore, as forested mountain streams they would be expected to have very low baseline nutrient concentrations (Harding *et al.* 1999). Lastly, leaf softening may have been affected by stream water pH as pectin-degrading enzyme activity is typically higher at high pH (Suberkropp & Klug 1980, Jenkins & Suberkropp 1995). The more rapid softening of leaves in the reference streams with pH 6.7 – 7.5 than in the flocculent (pH 5.5 – 6.8) and precipitate (pH 3.8 – 4.6) streams is consistent with this scenario, although the deposition of hydroxides in the latter group of streams makes classification of the main causal factor difficult to determine.

Finally, invertebrate taxonomic richness and the number of invertebrates per pack were generally higher at the reference sites than the precipitate sites although not significantly so, and no shredders were collected from precipitate sites. However, the density of shredders was low in leaf packs at all sites suggesting they had a negligible effect on leaf breakdown. As all the streams were in forest we might have expected that naturally occurring leaf packs would be common, however, their highly flood prone nature meant they were not, as also found by Cowie (1980) in adjacent Devils

Creek. In many West Coast streams breakdown of CPOM may be primarily by physical abrasion rather than microbial decomposition or processing by shredders, and the residence time of many leaves is likely to be short. Nevertheless, it is apparent from our study that precipitates slow the rate of leaf softening and thus appear to hinder the initial breakdown of leaves. The relative importance of this mechanism for leaf breakdown may be an issue for stream rehabilitation from mining impacts.

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